Acetate Metabolism by *Escherichia coli* in High-Cell-Density Fermentation

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Little is known about the cellular physiology of *Escherichia coli* at high cell densities (e.g., greater than 50 g [dry cell weight] per liter), particularly in relation to the cellular response to different growth conditions. *E. coli* W3100 cultures were grown under identical physical and nutritional conditions, by using a computer-controlled fermentation system which maintains the glucose concentration at 0.5 g/liter, to high cell densities at pH values of 6.0, 6.5, 7.0, and 7.5. The data suggest a relationship between the pH of the environment and the amount of acetate excreted by the organism during growth. At pH values of 6.0 and 6.5, the acetate reached a concentration of 6 g/liter, whereas at pH 7.5, the acetate reached a concentration of 12 g/liter. Furthermore, at pH values of 6.0 to 7.0, the *E. coli* culture undergoes a dramatic metabolic switch in which oxygen and glucose consumption and CO_2 evolution all temporarily decrease by 50 to 80%, with a concomitant initiation of acetate utilization. After a 30-min pause in which approximately 50% of the available acetate is consumed, the culture recovers and resumes consuming glucose and oxygen and producing acetate and CO_2 at preswitch levels. During the switch period, the specific activity of isocitrate lyase typically increases approximately fourfold.

It is well known that during rapid aerobic growth, strains of *Escherichia coli* produce acetic acid as a by-product (7, 9–14, 17, 19), although the amount of acetate produced is strain dependent (17). Luli and Strohl (17) found that more than a threefold difference in acetate concentrations may occur between different strains grown in batch fermentations under identical conditions. The growth rate of the culture and the composition of the medium also influence the amount of acetate produced during growth. In chemostat experiments, acetate is not produced until the growth rate reaches a threshold value that is dependent upon the type of growth medium (21). *E. coli* cultures will produce acetate at a lower growth rate when grown in a nutrient-rich medium than in a defined medium (9, 21), and the specific acetate production rate also changes with the growth rate (9).

Acetate is produced when the uptake of the primary carbon source is greater than its conversion to biomass and CO_2 (10). The rate-limiting aspect of metabolism contributing to acetic acid formation by *E. coli* has been attributed to the electron transport system (7), the tricarboxylic acid cycle (10), or a combination of both (19). In a batch culture growing on glucose, approximately 15% of the carbon input is typically excreted as acetate (10).

The presence of acetate in the growth medium can influence the physiology of the cell in many ways. Elevated acetate concentrations inhibit the growth rate of the culture, the effect of which may be more pronounced in complex medium than in defined medium (23). It has been postulated that the protonated form of acetate can cross the cytoplasmic membrane and reduce the proton motive force by decreasing ΔpH (7, 17), although recent evidence on acetate accumulation by *Streptococcus bovis* suggests that this proposed mechanism may not occur (27). High acetate concentrations are also known to decrease the amount of recombinant protein produced by *E. coli* (1, 18).

In fed-batch fermentations, the production of acetate is more significant than in batch fermentations because of the extended growth phase that allows the acetate to attain higher concentrations. Many fed-batch strategies have been developed recently with the specific purpose of preventing the accumulation of acetate (12, 14, 22, 25). These fed-batch methods typically result in lowered acetate concentrations through limitation of the culture for an essential nutrient such as the carbon-energy, oxygen, or nitrogen source. The effects of these limiting and perhaps starvation conditions on the cells and recombinant protein formation are not fully characterized.

With the development of a glucose predictive and feedback computer-controlled fermentation system (12), we are able to study the physiology of *E. coli* at high cell densities while maintaining the glucose at a low concentration without starving the cells. This system allows for the study of acetate metabolism at these high cell densities.

MATERIALS AND METHODS

Organism and inoculum preparation. *E. coli* W3100 (ATCC 14948) was maintained on Trypticase soy agar plates at 4°C between experiments. The seed cultures, consisting of 400 ml of Trypticase soy broth, were inoculated from the Trypticase soy agar plates and incubated for 18 h at 37°C with shaking at 200 rpm on a rotary shaker.

Fed-batch fermentation medium. The medium used during the fermentation was as described by Kleman et al. (12). Four feed solutions were used for fed-batch fermentation: (i) a 2.0-liter solution of glucose (70 g/liter); (ii) a 2.0-liter solution of glucose (375 g/liter) and $MgSO_4 \cdot 7H_2O$ (0.85 g/liter); (iii) a 2.0-liter solution of glucose (780 g/liter) and $MgSO_4 \cdot 7H_2O$ (8.58 g/liter); and (iv) a 1.0-liter solution of casein hydrolysate (CE90M; Delltown, Fraser, N.Y.) at 400 g/liter and trace elements (32 ml/liter). The calibration of the feed pumps and addition of the feed solutions to the vessel were as described by Kleman et al. (12). During the pump calibration, the density of the glucose feed solution was calculated by the following equation: density = $[(3.32 \times 10^{-4}) \cdot$ glucose concentration (grams per liter)] + 1.003.

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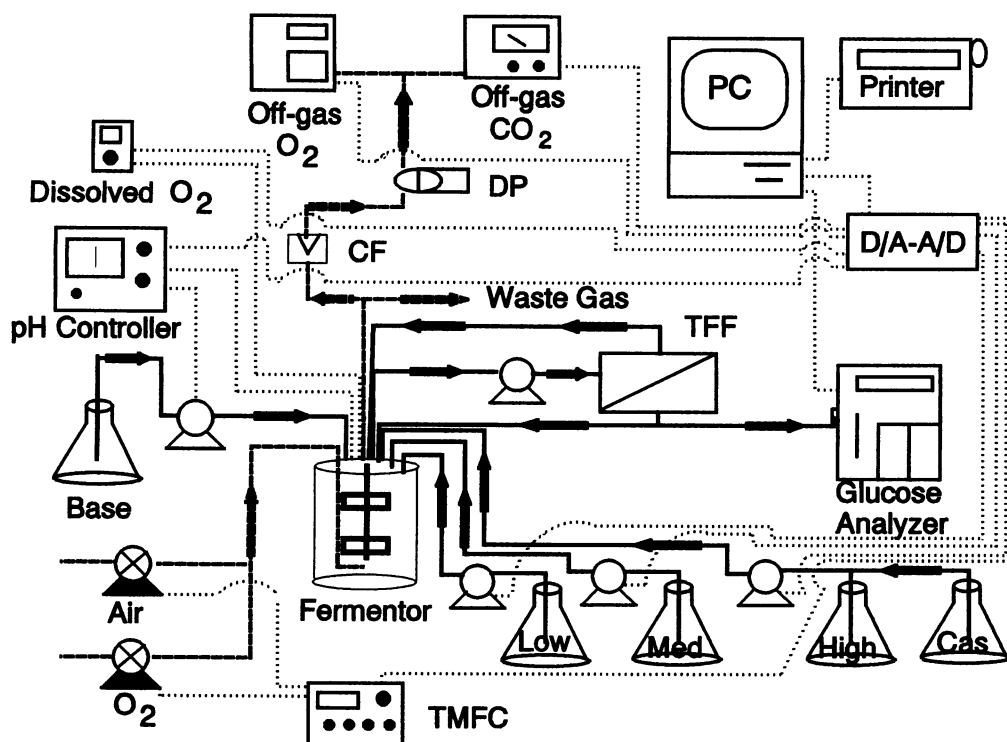
FIG. 1. Schematic of the computer-controlled fermentation system in which soluble glucose concentration in the fermentor was controlled by a combined predictive and feedback control scheme (12). The thin dashed lines represent electrical connections, while the thicker solid lines represent a flow of either fluid (solid lines) or gas (thick dashed lines). The arrows indicate the direction of flow. Symbols and abbreviations: ⬭, peristaltic pumps; ⬭, Tylan mass flow controllers; D/A, digital-to-analog converter; A/D, analog-to-digital converter; PC, IBM-compatible 386SX computer; DP, diaphragm pump; CF, coalescing filter; TMFC, Tylan mass flow controller. Three nutrient addition vessels are marked low, medium, and high, as defined in Materials and Methods; the hydrolyzed casein nutrient addition vessel is marked Cas.

Fermentation apparatus. A schematic of the fermentation system used for these experiments is shown in Fig. 1. The fermentor used was a modified 14-liter New Brunswick Scientific Microferm. The air control system, off-gas oxygen analyzer, off-gas carbon dioxide analyzer, and data acquisition hardware have been described previously (29). A diaphragm vacuum pump (Dynapump; Neptune, Dover, N.J.) was used to draw approximately 5 liters of off-gas per min through a coalescing filter (0.1-μm pore size; Balston, Haverhill, Mass.) to remove any water or particulate matter from the gas before it entered the off-gas analyzers. The diaphragm vacuum pump was used to prevent an increase of pressure within the fermentor vessel, a condition which interfered with proper operation of the Megaflow tangential-flow filtration device. The dissolved oxygen was monitored by an Ingold O_2 amplifier (type 170) and an Ingold polarographic oxygen probe. The tangential-flow system used for sampling by the glucose analyzer and the pH control system have been described previously (11–13).

The computer system used for monitoring and control consisted of an IBM-compatible 386SX computer operating at 16 MHz. An ISAAC 91A data acquisition and control device (Cyborg, Newton, Mass.) (29) was converted to work with an IBM-compatible computer. The program was written in Turbo Pascal version 6.0 (Borland International, Scotts Valley, Calif.) with a data acquisition library provided by Cyborg. The control scheme for maintaining a constant glucose concentration in the fermentor has been described by Kleman et al. (12).

The glucose analyzer used was a model 2700 biochemical analyzer (YSI Inc., Yellow Springs, Ohio) with a monitoring

station. Samples were taken from the filtrate side of the tangential-flow system and pumped to the sample chamber of the monitoring station. A purge time of 19 s was used to ensure that a fresh sample was being measured. A standard of 2.0 g of glucose per liter was used for internal calibration, which was carried out automatically every fifth sampling period. The glucose analyzer was connected to the computer system via an RS232 serial interface.

All of the fermentations described were performed with the same glucose feedback computer-controlled system and the optimized algorithm parameters (proportional constant $[K_c]$, 0.5; number of points used in determining the rolling linear regression, five) described previously (12).

The temperature of the fermentation cultures was controlled at 35°C. The total flow rate of gas into the fermentor was 10 liters/min under all conditions. The initial rate of agitation was 400 rpm, and this was increased manually during the early stages of the fermentations to 600 rpm to maintain dissolved oxygen (dO_2) at 70% relative to saturation. When a dO_2 of 70% was unachievable by using an agitation rate of 600 rpm and 10 liters of air per min, O_2 was added to the inlet gas as needed to maintain the set-point dO_2. dO_2 never decreased below 50% in any of the fermentations described herein.

The fermentations controlled at a soluble glucose concentration of 0.5 g/liter at pH 6.5 and 7.0 were carried out three and six times, respectively. For every other growth condition described, the fermentations for which reproducible data were obtained were carried out twice. For each experimental con-

dition, the data from a single representative fermentation are shown.

Culture analysis. Dry cell weight (DCW) was measured for each time point shown as described previously (11–13). Fermentation broth samples were prepared for acetic acid analysis by precipitation of macromolecules at pH 2.0 with an equal volume of 0.02 N H_2SO_4 at room temperature. The precipitate was pelleted for 2 min in a microcentrifuge, and the supernatants were filtered through a 0.2-μm-pore-size, 13-mm-diameter filter (Gelman, Ann Arbor, Mich.). The acetic acid produced during fermentation was quantitated by high-pressure liquid chromatography (HPLC). The system consisted of a Waters (Milford, Mass.) 600E multisolvent delivery system and a Waters U6K injector; stainless-steel or polyetheretherketone (PEEK) (Alltech Associates, Deerfield, Ill.) tubing was used throughout. An HPLC water jacket (Alltech Associates) and a circulating water bath (Lauda type K2; Brinkmann, Westbury, N.Y.) maintained the column temperature at 55°C. An organic acid analysis Aminex ion-exchange column (7.8 by 300 mm; model HPX-87H; Bio-Rad Laboratories, Richmond, Calif.) was used. Organic acids were separated by using a mobile phase of 0.01 N H_2SO_4 (pH 2.0) at a flow rate of 0.7 ml/min. The elution was monitored with a Waters 486 variable-wavelength spectrophotometer set at 210 nm, and the A_{210} was recorded and integrated with Waters Baseline 810 software running on a NEC 386SX computer with a 80387SX math coprocessor. Organic acid standards were prepared from reagent-grade chemicals dissolved in HPLC-grade water. Standards were injected under the same conditions as the fermentation samples, and the retention times were compared.

Enzyme and protein analysis. For enzyme analysis and protein determination, samples were taken from the fermentation and the cells were pelleted by centrifugation for 8 min at 8,000 × g (4°C). The supernatant was removed, and the pelleted cells were frozen immediately at −70°C until the analysis was performed. For analyses, the samples were thawed and resuspended in a buffer consisting of 64.5 mM KH_2PO_4-K_2HPO_4 and 6.45 mM $MgCl_2$ (pH 7.4). The cells were then lysed with an automated French pressure cell at 12,600 lb/in^2. Unbroken cells and cell walls were pelleted by centrifugation for 20 min at 20,000 × g (4°C), and the supernatants (crude cell extracts) were used for analyses. The protein concentration was determined by the dye-binding method using Coomassie brilliant blue G-250 (2); bovine serum albumin was used as the standard.

The specific activity of isocitrate lyase (ICL) in crude cell extracts of *E. coli* was measured by the phenylhydrazine method (6). The assay mixture consisted of the following components in a final volume of 1.0 ml: phosphate buffer, 64.5 μmol (pH 7.4); isocitrate, 10 μmol; $MgCl_2$, 10 μmol; cysteine-HCl, 5 μmol; and phenylhydrazine-HCl, 10 μmol. The reaction was started by the addition of cell extract. The reaction was monitored by measuring the change of A_{324} over time at 37°C. A molar extinction coefficient of 1.7 × 10^4 was used to convert absorbance to concentration (6).

RESULTS

Unless otherwise described, the fermentations were controlled by maintaining soluble glucose at 0.5 g/liter. Most of the experiments compare results of acetate formation and reconsumption by high-cell-density-grown *E. coli* cultures at different controlled pH values. In a final experiment, the effect of controlled glucose concentration on acetate formation and reutilization was determined. All of the fermentations were stopped when the feed pumps were no longer able to supply
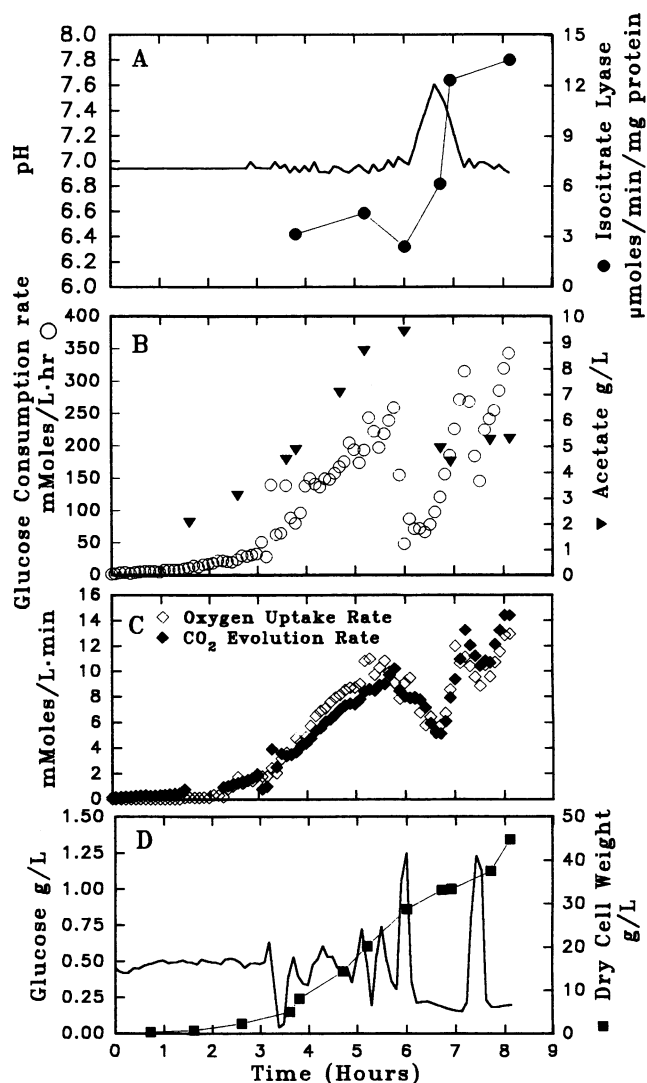

FIG. 2. Fed-batch fermentation of *E. coli* W3100 grown to high cell density with the pH controlled at 7.0 and the soluble glucose concentration controlled at 0.5 g/liter. (A) pH and the specific activity of ICL (in micromoles per minute per milligram of total soluble protein) with respect to fermentation time; (B) glucose consumption rate (in millimoles per liter per hour) and soluble acetate concentration in the fermentor (in grams per liter) with respect to time; (C) oxygen uptake and carbon dioxide evolution rates (both in millimoles per liter per minute) with respect to fermentation time; (D) glucose concentration (in grams per liter) and DCW (in grams per liter) with respect to fermentation time.

enough glucose to meet the glucose consumption requirements of the cultures.

Fermentation of *E. coli* W3100 at pH 7.0. In fed-batch cultures controlled at pH 7.0, a final DCW of 45 to 50 g/liter was obtained in about 8 to 9 h (Fig. 2). During the exponential growth period (hours 0 to 5), the growth rate (μ; 0.91 h^{-1}), glucose consumption rate (1.035 g/liter · h), and acetate production rate (0.39 g/liter · h) were constant (Fig. 3).

The rates of acetate production and glucose consumption, however, were considerably different, which was unexpected. On a per DCW basis, the specific glucose consumption rate was roughly constant at 2.3 g of glucose consumed per g
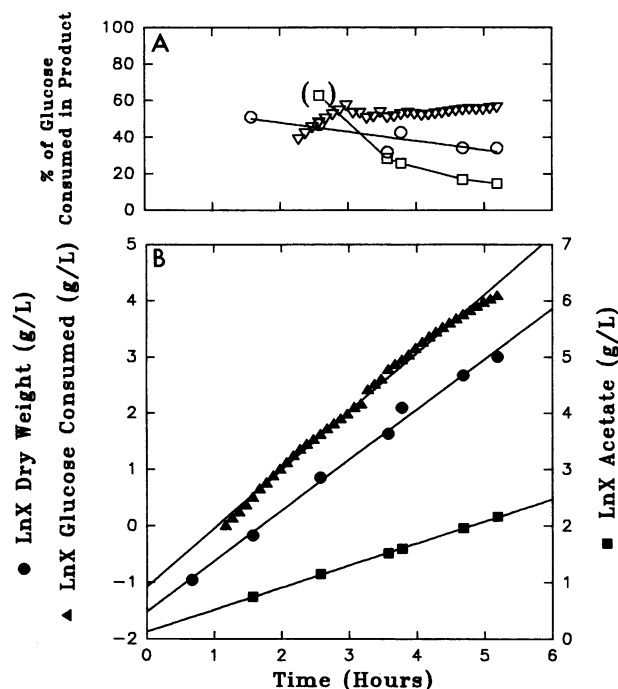
FIG. 3. (A) A plot of biomass (O), acetate (□), and CO_2 (∇) as percentages of glucose consumed as a function of fermentation time. These data are calculated from the data presented in Fig. 2. (B) Plot of the natural log (LnX) values of cumulative glucose consumption (in grams per liter), giving a rate of 1.035 g/liter · h, DCW (in grams per liter), giving a specific growth rate (μ) of 0.91 h^{-1}, and acetate production (in grams per liter), giving a rate of 0.39 g/liter · h, during the initial exponential growth phase of the culture. The glucose consumption data were obtained from computer data used to determine glucose consumption rate, and the biomass and acetate data are calculated and replotted from Fig. 2.

(DCW), giving a calculated average mass yield from glucose of 0.43 during the initial exponential phase (Fig. 3). The specific acetate production rate, however, decreased in a constant proportion from approximately 0.9 g of acetate produced per g (DCW) at 3 h to 0.3 g/g (DCW) at 8 h (calculated from data shown in Fig. 3). Thus, as compared with the flow of ca. 15% of carbon from glucose to acetate observed in batch cultures (10), a range of 13 to 30% of the glucose consumed was excreted as acetate in the high-cell-density cultures during most of the exponential phase. Early values for acetate production as a fraction of glucose consumed were consistently higher than expected (Fig. 3) because of unknown factors. Although the acetate concentration in the fermentor increased at a relatively constant rate, the production of acetate per unit DCW decreased as the fermentation progressed, suggesting that there is a dynamic regulation of acetate production as the cultures become denser, up to the point where a metabolic switch takes place, as described later. It appears that this dynamic regulation of acetate production during exponential growth may be based on the amount of acetate in the medium since nothing else, other than the DCW, in the culture changes appreciably during this period.

Soluble glucose concentration was maintained steady until 3.5 h, at which time a short-lived drop occurred (Fig. 2D) as a result of initiation of the third feed pump which operates at higher revolutions per minute than the first two pumps. At low revolutions per minute, the third pump requires a longer time

to achieve the proper speed, which results in a slight underfeeding of the culture. This anomaly was present in every fermentation run.

At approximately 6 h of growth, at which time the culture density was at ca. 30 g (DCW) per liter and acetate had reached ca. 9.5 g/liter, the glucose and oxygen consumption rates and CO_2 evolution rate suddenly and reproducibly decreased (Fig. 2B and C). This sudden reduction of glucose requirement always occurred at a rate higher than that to which the control algorithm could respond, resulting in a marked momentary increase in the glucose concentration (Fig. 2D). We have termed this period of the culture, whereby the metabolism appears to pause for a period of ca. 40 min, the switch. Shortly after the dissipation of glucose consumption, the pH (Fig. 2A) of the culture increased as a result of the consumption of acetate from the medium (Fig. 2B). Approximately 40 min after the initial sudden decrease in glucose and oxygen consumption rates, consumption of glucose and consumption of acetate resumed together until the acetate concentration reached approximately half of the maximum concentration observed, at which time acetate consumption waned. The metabolism of the culture then returned (at hour 7) to that of normal aerobic growth, wherein glucose was consumed and acetate was produced, albeit acetate production was at a much lower rate this time. The pH of the culture decreased to the original set point at this point.

During the period when both glucose and acetate were consumed, starting at about 6.5 h, a ca. fourfold increase in the specific activity of ICL, the initial enzyme of the glyoxylate shunt pathway, was observed. The increase in activity of this enzyme correlated directly with the consumption of acetate after the switch.

Fermentation of *E. coli* W3100 at pH 6.5. The results for the glucose-controlled fermentation maintained at pH 6.5 are shown in Fig. 4. The initial growth rate of the culture was 1.0 h^{-1} (Fig. 4D). As with the fermentation controlled at pH 7.0, the glucose and oxygen consumption and the CO_2 evolution rates correlate directly with the increase in DCW during exponential growth (11). At ca. 6 h into the fermentation, when DCW and acetate had reached concentrations of ca. 23 and 6 g/liter, respectively, a sudden decrease in glucose consumption was observed, as expected from the results of the previous fermentations at pH 7.0. The pH 6.5-controlled culture began to consume acetate (Fig. 4B), resulting in an increase in pH. Once the culture recovered and the acetate concentration had dropped from ca. 6 to 3.5 g/liter, glucose consumption increased very quickly and the culture began to produce acetate again, as in the fermentations carried out at pH 7.0.

Unlike the fermentations at pH 7.0, however, a second sudden decrease in glucose consumption was observed approximately 2 h later together with a concomitant rise in pH and decrease in acetate concentration. Interestingly, the acetate concentration at the beginning of this second switch was 4.7 g/liter, less than the 6 g/liter present at the point where the first switch took place. The recovery time for the cultures after the second switch also was longer than the recovery time observed after the first switch. Again, oxygen consumption and CO_2 evolution (Fig. 4C) mirrored the glucose consumption data. The specific activity of ICL increased fourfold during the initial switch at 7 h into the fermentation; however, ICL specific activity remained elevated during the subsequent cycles of acetate production and consumption (Fig. 4A).

Fermentation of *E. coli* W3100 at pH 6.0. Glucose-controlled fermentations of *E. coli* maintained at pH 6.0 were, in the initial stages, similar to those described above (11). At pH 6.0, the initial specific growth rate was comparable to that of the
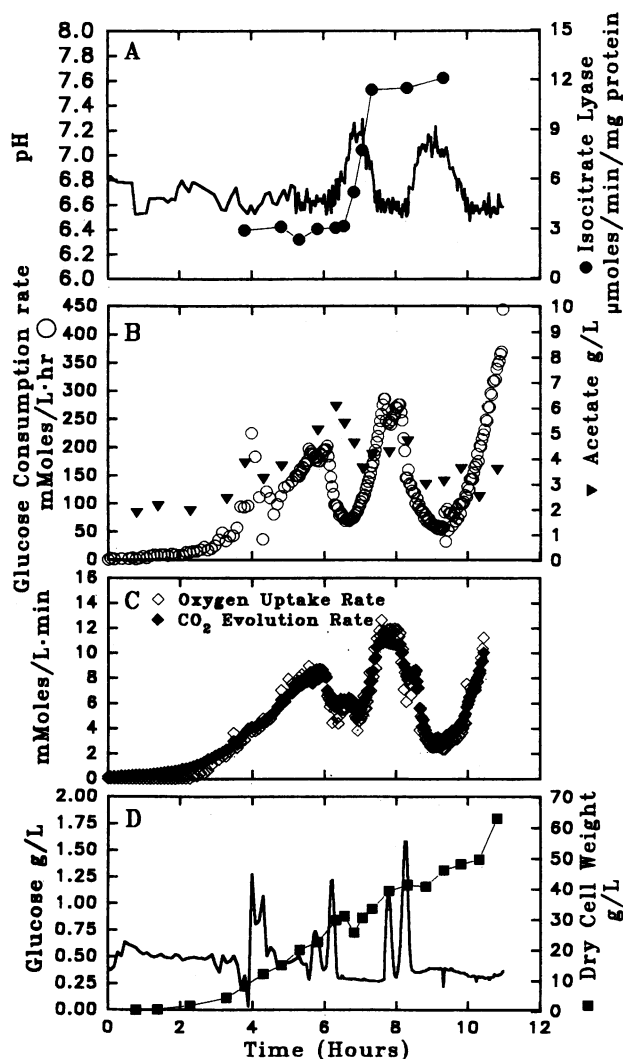
FIG. 4. Fed-batch fermentation of *E. coli* W3100 grown with the pH controlled at 6.5 and the glucose concentration controlled at 0.5 g/liter. (A) pH and the specific activity of ICL versus time; (B) glucose consumption rate and acetate concentration versus time; (C) oxygen uptake rate and carbon dioxide evolution rate versus time; (D) glucose concentration and DCW versus time. All values are as described in the legend to Fig. 2.

previously described fermentations (0.93 h^{-1}), and the glucose consumption, oxygen consumption, and CO_2 evolution rates correlated directly with the specific growth rate. In these fermentations, however, when the DCW and total acetate concentration reached 11 and 5.5 g/liter, respectively, glucose consumption decreased rapidly, as if the switch were occurring, but the cultures did not recover (11). Shortly thereafter, the glucose and oxygen consumption rates fell to zero, indicating that the culture was dead. The specific activity of ICL did not change throughout the self-aborted fermentations that occurred at pH 6.0.

Fermentation of *E. coli* W3100 at pH 7.5. Fermentations controlled at pH 7.5 had slightly lower growth rates (0.75 h^{-1}) but resulted in a higher final biomass (71 g [DCW] per liter) (11). Total acetate concentration, which reached 15 g/liter at 9 h into the fermentations, did not appear to affect the growth of

the organism. A slight dip in glucose consumption appeared when the acetate concentration attained 11.2 g/liter, but the culture grew steadily thereafter, albeit at a lower specific growth rate (0.4 h^{-1}), until the end of the fermentation. During this lower growth rate period, acetate was slowly utilized to a concentration of 7.8 g/liter at the end of the fermentation. The specific activity of ICL was approximately 4 μmol/min/mg of protein throughout the fermentations at pH 7.5 and did not change appreciably at any point in the fermentations (11).

Fermentation of *E. coli* W3100 at pH 6.5 and glucose at 2.0 g/liter. To determine if the acetate production-consumption switch was influenced by the level of controlled glucose concentration, fermentations were controlled at pH 6.5 (the pH at which we observed multiple switches) and the glucose concentration was controlled at 2.0 g/liter. The first 5 h of these fermentations, with an initial specific growth rate of 0.985 h^{-1}, were very similar to those of the corresponding fermentations at 0.5 g of glucose per liter (Fig. 5). Shortly before 6 h into the fermentation, when the acetate concentration reached 7.2 g/liter, a sharp decrease (40% reduction in 10 min) in the glucose consumption rate similar to that described above for the fermentations performed with glucose controlled at a concentration of 0.5 g/liter was observed. In the fermentations controlled at 2.0 g of glucose per liter, however, the culture did not consume acetate during this period of decreased glucose consumption. After 40 min, the culture recovered and continued with a combined increase in glucose consumption and additional acetate production. During this entire time of decreased glucose consumption, neither the pH nor the specific activity of ICL increased, indicating that appreciable consumption of acetate did not occur at the time of the switch under these cultural conditions.

DISCUSSION

Characteristics of the switch. Cultures of *E. coli* W3100 growing in our glucose predictive and feedback-controlled fermentation system (11, 12) undergo an interesting pause and switch in metabolism midway through the fermentation. During the initial exponential growth period, acetate is produced by the cultures, which is typical of *E. coli* cultures growing on glucose, to levels considered to be toxic to cell growth (7, 9–14, 17, 23). At a point toward the end of exponential growth, when acetate concentrations reach their peak levels, the glucose and oxygen consumption and CO_2 production rates rapidly decrease in a 20-min time period to nearly 50% of their maximal levels. Concomitant with these drastic decreases in glucose and oxygen consumption, the fermentations controlled at a concentration of 0.5 g of soluble glucose per liter is the initiation of acetate utilization, which continued until approximately half of the acetate was consumed. Finally, glucose consumption increases again and the culture begins to produce acetate again. The acetate concentration in the growth medium may be linked to this phenomenon of decreasing glucose consumption rate.

We have observed this switch phenomenon in glucose-controlled high-cell-density cultures (glucose controlled at 0.5 g/liter; pH 7.0) of *E. coli* W3110 (Fig. 2 and 4), MV1190 (Bio-Rad, Hercules, Calif.) (11), RB791 (11), and K-12 (ATCC 10798) (11). Each one of these strains produced at least 8 g of acetate per liter under fermentation conditions similar to those shown in Fig. 2 (11). Moreover, fermentation profiles of these other strains were similar in each case to that shown in Fig. 2 (11).

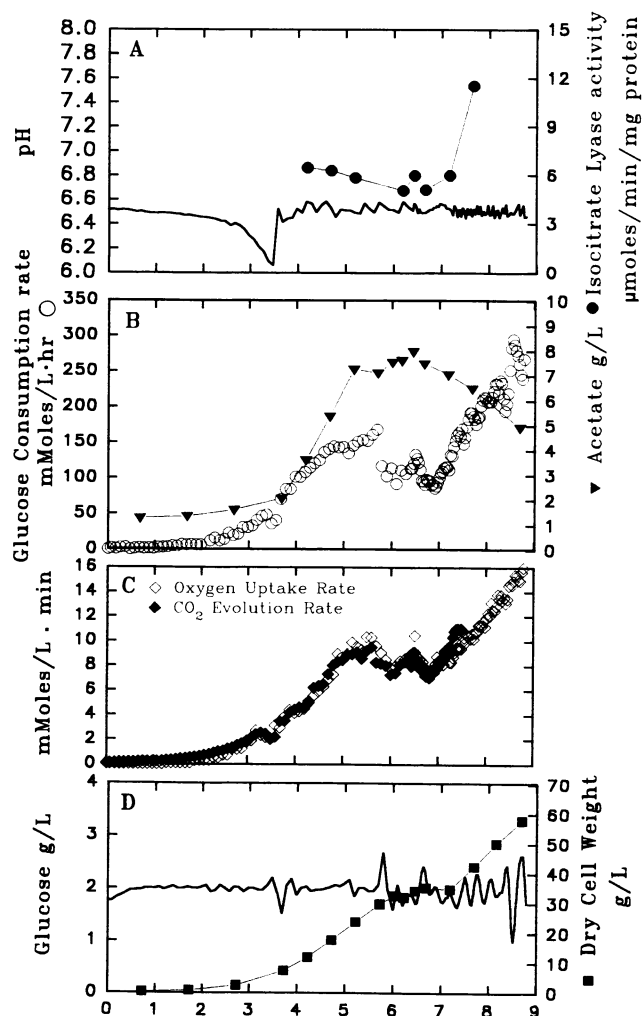We did not observe this phenomenon with our earlier

FIG. 5. Fed-batch fermentation of *E. coli* W3100 grown with the pH controlled at 6.5 and the glucose concentration controlled at 2.0 g/liter. (A) pH and the specific activity of ICL versus time; (B) glucose consumption rate and acetate concentration versus time; (C) oxygen uptake rate and carbon dioxide evolution rate versus time; (D) glucose concentration and DCW versus time. All values are as described in the legend to Fig. 2.

experiments utilizing the same fermentation system growing *E. coli* B (OSU 333) (11, 12). *E. coli* B, however, produced only low amounts of acetate during exponential growth (ca. 2 to 3 g of acetate per liter, even with cell densities reaching 70 g [DCW] per liter) (11, 12). This is likely due to a mutation in one of the two enzymes required for acetate production, phosphotransacetylase or acetate kinase (7), as shown by the ability of *E. coli* B to grow on nutrient agar plates containing fluoroacetate (11, 16).

Effect of glucose concentration on acetate consumption. The soluble glucose concentration in the vessel appears to affect acetate consumption after the switch. At constant low glucose levels (0.3 to 0.5 g/liter), acetate utilization began almost immediately after the decrease in glucose consumption rate, and the specific activity of ICL increased fourfold. At a controlled glucose concentration of 2.0 g/liter, the decrease in glucose consumption occurred as in other fermentations controlled at 0.5 g/liter, but acetate was not utilized and the

specific activity of ICL did not increase until well after the switch. This suggests that at higher glucose concentrations, acetate utilization was inhibited even though the apparent cause of the switch, i.e., high acetate concentration, was still present. The K_s (saturation constant) of *E. coli* for glucose is 4 mg/liter (24), a level considerably below either the 0.5 or 2.0 g/liter used in these experiments. Thus, both of the controlled glucose concentrations (e.g., 0.5 and 2.0 g/liter) were saturating with respect to the specific growth rate of *E. coli*. This suggests that an unknown effect different from one that is growth rate related controls whether acetate is consumed in these high-cell-density cultures.

Increase of ICL activity. The approximate fourfold increase of ICL activity after the sudden decrease of the glucose consumption rate indicates that the glyoxylate shunt pathway was turned on specifically under the conditions shown (i.e., constant level of 0.5 g of glucose per liter and accumulation of 6.0 g of acetate [pH 6.5] per liter or ca. 10 g of acetate [pH 7.0] per liter). The mechanism for this activity increase, either by induction of the gene for this enzyme or activation of the enzyme itself, is not known. The genes for the glyoxylate shunt pathway (malate synthase, ICL, and isocitrate dehydrogenase kinase/phosphatase) are clustered and transcribed on a single transcript (4). The promoter for this transcript is regulated by IclR, a repressor that binds to the −35 region to prevent transcription (5). Phosphoenolpyruvate appears to prevent IclR binding to the promoter, but no effect on IclR binding was observed with acetate, acetyl-coenzyme A, pyruvate, oxaloacetate, and acetyl-phosphate as corepressors (5). Although phosphoenolpyruvate may derepress the transcription of the glyoxylate shunt pathway enzymes, it is also a noncompetitive inhibitor of ICL (15). Phosphorylation of ICL appears to be required for the enzyme to be active (26). Our data suggest that either the enzyme is present previously and activated by phosphorylation under the specific conditions where ICL activity quickly rises or transcription of the *ace* operon is derepressed.

Possible mechanisms for the switch. The actual mechanism for the switch to acetate utilization while glucose is present is not known. The factors that appear to play a role include acetate concentration, pH, glucose concentration, and induction or activation of ICL. Possible mechanisms for this switch may include induction of gene expression by the accumulated acetate (28, 31), interaction of phosphorylated acetate kinase with the phosphotransferase system for glucose transport (8), and/or cross-talk signaling mediated by acetyl-PO_4, a key intermediate in acetate metabolism (3), which is known to interact with several metabolic pathways (20, 30).

The growth of *E. coli* to high cell densities at constant low-glucose concentrations allows for the study of cellular metabolism that may not be observed in normal batch fermentations or other fed-batch systems where starvation of the culture may occur. In our system, the external acetate concentration appears to cause an effect whereby glucose consumption decreases and then acetate is utilized until the acetate concentration decreases to a noneffective concentration. At that point, the culture again consumes glucose and produces acetate. This effect seems to be related to the glucose concentration of the growth environment because acetate reutilization coincident with the switch was not observed when the glucose concentration was controlled at 2.0 g/liter.

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